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(71) Applicant(s)
Merck & Co Inc
(Incorporated in USA - New Jersey)
PO Box 2000, Rahway, New Jersey, 07065-0900,
United States of America

(72) Inventor(s)
Eliav Barr
Robert J Gould
Kenneth A Thomas Jr

(74) Agent and/or Address for Service
Merck & Co Inc
European Patent Department, Terlings Park,
Eastwick Road, HARLOW, Essex, CM20 2QR,
United Kingdom

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(54) Abstract Title
Treatment of coronary and/or peripheral ischemia using an antithrombotic agent and an angiogenesis promoter

(57) A method for treating a patient having coronary and/or peripheral ischemic syndrome by administering to the patient an effective amount of an antithrombotic agent, e.g. a glycoprotein IIb/IIIa antagonist, a thrombin inhibitor, a factor Xa inhibitor, or a low molecular weight heparin, and an effective amount of an angiogenesis promoter, e.g. a vascular endothelial growth factor or a fibroblast growth factor. Administration of the angiogenesis promoters may be by any suitable means known to persons skilled in the art, e.g. via bolus injection, continuous intravenous administration, coated stent implantation and gene transfer, which provides localized or systemic delivery of the angiogenesis promoter at the ischemic tissue.

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TITLE OF THE INVENTION
TREATMENT OF CORONARY AND PERIPHERAL ISCHEMIA USING AN ANTITHROMBOTIC AGENT AND AN ANGIOGENESIS PROMOTER

BACKGROUND OF THE INVENTION

Platelet activation and aggregation are central to the pathophysiology involved in unstable angina and acute myocardial infarction, in reocclusion following thrombolytic therapy and angioplasty, in transient ischemic attacks and in a variety of other vaso-occlusive disorders. When a blood vessel is damaged either by acute intervention such as angioplasty, or, more chronically, by the pathophysiological processes of atherosclerosis, platelets are activated to adhere to the disrupted surface and to each other. This activation, adherence and aggregation may lead to occlusive thrombus formation in the lumen of the blood vessel.

The final obligatory step in platelet aggregation is the binding of fibrinogen to an activated membrane-bound glycoprotein complex, GP IIb/IIIa ($a_{II}b_3$). Platelet activators such as thrombin, collagen, epinephrine or ADP, are generated as an outgrowth of tissue damage. During activation, GP IIb/IIIa undergoes changes in conformation that results in exposure of occult binding sites for fibrinogen. There are six putative recognition sites within fibrinogen for GP IIb/IIIa and thus fibrinogen can potentially act as a hexavalent ligand to crossing GP IIb/IIIa molecules on adjacent platelets. A deficiency in either fibrinogen or GP IIb/IIIa prevents normal platelet aggregation regardless of the agonist used to activate the platelets. Since the binding of fibrinogen to its platelet receptor is an obligatory component of normal aggregation, GP IIb/IIIa is an attractive target for an antithrombotic agent.

The monoclonal antibody 7E3, which blocks the GP IIb/IIIa receptor, has been shown to be an effective therapy for the high risk angioplasty population. It is used as an adjunct to percutaneous transluminal coronary angioplasty or atherectomy for the prevention of acute cardiac ischemic complications in patients at high risk for abrupt closure of the treated coronary vessel.

A study reported in The New England Journal of Medicine, 1994; 330: 956-961 showed a decrease from 12.8% to 8.3% in the combined endpoints of death, non-fatal MI and need for urgent revascularization with fibrinogen receptor blockade. This benefit was at the expense of some additional risk of bleeding, with the need for transfusion increasing from 3% to 6%, and the incidence of patients with decreased hematocrit increasing from 7% to 15%. 7E3 was added to the standard regime of heparin and aspirin thus leaving few hemostatic control mechanisms intact. The clinical benefits of this drug could be seen at 6 months.

Many other studies have shown that blocking the GPIIb/IIIa receptor will stop platelet aggregation induced by all of the agonists and thus prevent thrombus formation but leave platelet adhesion relatively intact. The 7E3 monoclonal antibody is described in Coller et al., Ann. NY Acad. Sci. USA, 1991; 614: 193-213; and Coller et al., J. Clin Invest., 1985; 76: 101-108. Others have used agents based on the RGD sequence, including snake venom proteins, small peptides, and peptidomimetics (Cook et al., Drugs of the Future, 1994; 19: 135-159; and Cox et al., Medicinal Research Reviews, 1994; 14: 195-228).

The results of the 7E3 study support the hypothesis that blockade of GPIIb/IIIa receptors is useful in preventing platelet thrombi. They also support the hypothesis that platelet-dependent thrombi frequently contribute significantly to the development of ischemic complications after PTCA, even when minor mechanical dissections are present.

The formation of new blood vessels either from differentiating endothelial cells during embryonic development (vasculogenesis) or from pre-existing vessels during adult life (angiogenesis) is an essential feature of organ development, reproduction, and wound healing in higher organisms. Folkman and Shing, J. Biol. Chem., 267: 10931-10934 (1992); Reynolds et al., FASEB J., 6: 886-892 (1992); Risau et al., Development, 102; 471-478 (1988). Angiogenesis is also necessary for certain pathological processes including tumorigenesis (Folkman, Nature Medicine, 1: 27-31 [1995]) and retinopathy, Miller et al., Am. J. Pathol., 145: 574-584 (1994). Known

angiogenesis promoters include vascular endothelial growth factors such as VEGF-A (mature isoforms containing 206, 189, 165, 145 and 121 amino acid residues), the individual VEGF-A isoforms, VEGF homologues including VEGF-B, VEGF-C, VEGF-D and placenta growth factor (PlGF), heterodimers composed of homologous VEGF subunits such as VEGF-A and either VEGF-B, VEGF-C, VEGF-D or PlGF, and fibroblast growth factors such as acidic fibroblast growth factor (including stabilized mutated Ser 117 form of acid fibroblast growth factor) and basic fibroblast growth factor, either with or without an artificial N-terminal secretory polypeptide sequence, and other factors (for example, hepatocyte growth factor (HGF)).

SUMMARY OF THE INVENTION

A method for treating a patient having coronary or peripheral ischemic syndrome by administering to the patient an effective amount of an antithrombotic agent, e.g., a glycoprotein IIb/IIIa antagonist, a thrombin inhibitor, a factor Xa inhibitor, tissue factor pathway inhibitors, thrombin receptor antagonists, or a low molecular weight heparin, and an effective amount of an angiogenesis promoter, e.g. a vascular endothelial growth factor or a fibroblast growth factor. Administration of the angiogenesis promoters may be by any suitable means known to persons skilled in the art, e.g. via bolus injection, continuous intravenous administration, coated stent implantation and gene transfer, which provides localized delivery of the angiogenesis promoter at the ischemic tissue

DETAILED DESCRIPTION OF THE INVENTION

The invention is a method for treating a patient having coronary or peripheral ischemic syndrome by administering to the patient an effective amount of an antithrombotic agent, e.g. a glycoprotein IIb/IIIa antagonist, a thrombin inhibitor, a factor Xa inhibitor, or a low molecular weight heparin, by typical means of administration including oral, intravenous, intramuscular, subcutaneous or ocular administration, and an effective amount of an angiogenesis promoter, e.g. a vascular endothelial growth factor such

as VEGF-A (mature isoforms 206, 189 165, 145, and 121 amino acid residues), VEGF 206, VEGF 189, VEGF 165, VEGF 145, VEGF 121, VEGF 120, VEGF homologues including VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF), VEGF heterodimers such as those composed of a VEGF-A subunit and a VEGF homologue (VEGF B (forming VEGF A/VEGF B heterodimer), VEGF C (forming VEGF A/VEGF C heterodimer), VEGF D (forming VEGF A/VEGF D heterodimer), or placenta growth factor (PlGF)(forming VEGF A/PlGF heterodimer)), a fibroblast growth factor such as acidic fibroblast growth factor (aFGF, or FGF-1), modified aFGF such as aFGF Ser 117, basic fibroblast growth factor (bFGF, or FGF-2), and FGF family members including FGF-3, FGF-4, FGF-5, FGF-6, FGF-8 and FGF-9 that bind to FGF receptors present on vascular endothelial cells, promoting their mitosis and/or migration, and FGF-10, FGF-11, FGF-12, FGF-13, FGF-14 and FGF-15, a platelet derived growth factor (PDGF AA, AB, or BB), a hepatocyte growth factor (HGF), or an insulin-like growth factor (e.g. IGF-I or IGF-II), by typical means of angiogenesis promoter administration including any suitable means known to persons skilled in the art, e.g. direct protein addition such as by bolus injection, continuous intravenous administration, coated stent implantation, or genetic delivery using modified viral or non viral vector (such as liposome/DNA complexes, "naked DNA", or adenoviruses) that enter via receptor-mediated gene transfer modification of the vector or virus for cell receptor specificity which leads to endocytosis, e.g., pinocytosis or phagocytosis, whereby the angiogenesis promoter is produced and then taken into a cell by invagination of the plasma membrane, which it breaks off as a boundary membrane of the part engulfed, which angiogenesis promoter administration provides localized delivery of the angiogenesis promoter at the ischemic tissue, such that the beneficial therapeutic effect of the antithrombotic agent is realized by the patient at substantially the same time as the beneficial effect of angiogenesis promoter.

The invention is also a method for reducing the risk of acute coronary ischemic syndrome in patients at risk to acute coronary ischemic syndrome comprising administering to the patient an effective

amount of an antithrombotic agent and an effective amount of an angiogenesis promoter. Patients at risk include those who suffer initial coronary ischemic syndrome symptoms and who are therefore more likely than others who have not suffered such symptoms to experience further thrombosis and ischemic tissue damage. The method of the present invention provides the patient with a reduced likelihood of further clot formation while enhancing tissue repair.

The invention is also the use of an antithrombotic agent, in combination with the use of an angiogenesis promoter, in the manufacture of a medicament effective for treating coronary ischemia.

Glycoprotein IIb/IIIa antagonists

Antagonists for the glycoprotein IIb/IIIa fibrinogen receptor have been described in United States Patents 5,470,849, 5,463,011, 5,455,243, 5,451,578, 5,446,056, 5,441,952, 5,422,249, 5,416,099, 5,405,854, 5,397,791, 5,393,760, 5,389,631, 5,380,713, 5,374,622, 5,358,956, 5,344,783, 5,340,798, 5,338,7235,334,596, 5,321,034, 5,318,899 (e.g. cyclic heptapeptides Mpr-(Acetimidyl-Lys)-Gly-Asp-Trp-Phe-Cys-NH_2, Mpr-(Acetimidyl-Lys)-Gly-Asp-Trp-Phe-Pen-NH_2, Mpr-(Phenylimidyl-Lys)-Gly-Asp-Trp-Phe-Pen-NH_2,and Mpr-(Phenylimidyl-Lys)-Gly-Asp-Trp-Phe-Cys-NH_2, wherein Mpr is mercapto propionyl), 5,312,923, 5,294,616, 5,292,756, 5,281,585 5,272,158, 5,264,420, 5,260,307, 5,239,113 (e.g. Ethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentynoate), 5,227,490, 5,206,373, 4,703,036 (e.g. N-Methyl-D-phenylalanyl-N-[(1S)-1-formyl-4-guanidinobutyl]-L-prolinamide), EP 505 868 (e.g. ((1-(2-((4-(aminoiminomethyl)benzoyl)amino)-3-(4-hydroxyphenyl)-1-oxopropyl)-4-piperidinyl)oxy)-(S)-acetic acid), WO 9311152 (e.g. N-(2-(2-(((3-((aminoiminomethyl)amino)propyl)amino)-carbonyl)-1-piperidnyl)-1-(cyclohexylmethyl)-2-oxoethyl)-(R,S)-glycine), EP 333 356 and WO 9422820, WO 95/14683, and WO 94/18981, all of which are herein incorporated by reference, and wherein the scope of this invention includes, but is not limited to, the use of each of the specifically disclosed compounds therein. They are described as useful for inhibiting fibrinogen binding and inhibiting clot formation.

In particular, the GP IIb/IIIa receptor antagonist is selected from: [3(R)-[2-piperidin-4-yl)ethyl]-2-piperidone-1]acetyl-3(R)-methyl-b-alanine) described in United States Patent No. 5,281,585; 2(S)-[(p-Toluenesulfonyl)amino]-3-[[[5,6,7,8-tetrahydro-4-oxo-5-[2-(piperidin-4-yl)ethyl]-4H-pyrazolo-[1,5-a][1,4]diazepin-2-yl]carbonyl]-amino]propionic acid described in WO 94/18981; 5-[(4-Piperidinyl)methoxy]-2-indolecarbonyl-2(S)-phenylsulfonyl-amino-b-alanine described in WO 97/15568; and 2-S-(n-Butylsulfonylamino)-3[4-piperidin-4-yl)butyloxyphenyl]propionic acid hydrochloride (also known as tirofiban) described in United States Patent 5,292,756; DMP 728, described in Circulation, 1996 93 :537-543, Cox et al., Medicinal Research Reviews, 1994, 14 :195-228, and Cook et al., Drugs of the Future, 1994, 19(2) :135-159, from DuPont Merck; DMP 754 ((R)-methyl-3-[[[3-[4-(aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-N-(butoxycarbonyl)-L-alanine monoacetate, described in WO 95/14683) from DuPont-Merck; Ro44-9883 and Ro43-8857, both described in Cook et al., ibid, and Cox et al., ibid, from Hoffman-LaRoche; xemlofiban (also known as xemilofiban) from Searle/Sankyo, described in *Circulation*, 1995, *92* :2331; fradafiban from Boehringer Ingleheim/K. Thomae; SB 2144857 (from SmithKline Beecham); ZD2486 (from Zeneca); TAK 029, described in *J. Pharmacology and Experimental Therapeutics*, 1996, 277 :502-510, from Takeda; orbofiban and SC-58635 from Searle; SC54684, described in Cook et al., ibid, and Cox et al., ibid, from Searle; GR144053, described in Thrombosis and Hematosis, 1993, 69 :1071, Cook et al., ibid, and Cox et al., ibid, from Glaxo; compound 109891 from Rhone Polenc Rorer; and sibrafiban from Hoffman-LaRoche as described in EP 656348.

Glycoprotein IIb/IIIa receptor antagonists and their pharmaceutically acceptable salts are useful in the present invention. The term "pharmaceutically acceptable salts" means non-toxic salts of the compounds (which are generally prepared by reacting the free acid with a suitable organic or inorganic base) which include, but are not limited to, acetate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, calcium edetate, camsylate, carbonate, chloride, clavulanate, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate,

glycollylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynapthoate, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylbromide, methylnitrate, methylsulfate, mucate, napsylate, nitrate, oleate, oxalate, pamaote, palmitate, panthothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide, valerate.

Pharmaceutically effective amounts of the glycoprotein IIb/IIIa receptor antagonists are suitable for use in the methods of the present invention. The term "pharmaceutically effective amount" means that amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system or animal that is being sought by a researcher or clinician.

The compositions and methods of the present invention are useful in combination with procedures for treating patients with other anticoagulants (e.g. thrombin inhibitors such as heparin, Factor Xa inhibitors such as warfarin, tissue factor pathway inhibitors, or thrombin receptor antagonists), thrombolytic agents (e.g. streptokinase and tissue plasminogen activator), and platelet antiaggregation agents (e.g. aspirin and dipyridamole).

In accordance with the invention, glycoprotein IIb/IIIa receptor antagonists can be administered to the patient in an oral composition such as a tablet or capsule, in an ocular formulation, or in an intravenous solution. Administrations in these various ways are suitable for the present invention as long as the beneficial therapeutic effect of the glycoprotein IIb/IIIa receptor antagonist is realized by the patient at substantially the same time as the beneficial effect of growth factors. For purposes of this application, beneficial therapeutic effect includes both the desired patient response and initiation of conditions in the patient providing for the desired patient response.

Suitable oral compositions include tablets, capsules (each of which includes sustained release or timed release formulations), pills, powders, granules, elixirs, tinctures, suspensions, syrups, and emulsions. Suitable intravenous compositions include bolus or extended

infusion. Such oral and intravenous compositions are well known to those of ordinary skill in the pharmaceutical arts.

The active drugs may be administered to patients where prevention of thrombosis by inhibition of binding of fibrinogen to the platelet membrane glycoprotein complex IIb/IIIa receptor is desired. Such administration is useful in surgery on peripheral arteries (arterial grafts, carotid endarterectomy) and in cardiovascular surgery where manipulation of arteries and organs, and/or the interaction of platelets with artificial surfaces, leads to platelet aggregation and consumption. The aggregated platelets may form thrombi and thromboemboli. The active drug may be administered to these surgical patients to prevent the formation of thrombi and thromboemboli. The active drugs may also be administered to treat stroke, carotid percutaneous transluminal coronary revascularization, or carotid endarterectomy.

Other applications of the drugs includes prevention of platelet thrombosis, thromboembolism and reocclusion during and after thrombolytic therapy and prevention of platelet thrombosis, thromboembolism and reocclusion after angioplasty or coronary artery bypass procedures, or to improve outcomes following stent implantation (i.e., to prevent thromboembolism on device insertion). It may also be used to treat patients with unstable angina and prevent subsequent myocardial infarction.

The dosage regimen utilizing the active drugs is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular compound or salt thereof employed. An ordinarily skilled physician or veterinarian can readily determine and prescribe the effective amount of the drug required to prevent, counter, or arrest the progress of the condition.

Oral dosages of active drug when used for the indicated effects, will range between about 0.005 mg per kg of body weight per day (mg/kg/day) to about 50 mg/kg/day and preferably 0.005-20 mg/kg/day and most preferably 0.005-10 mg/kg/day. Suitable oral tablets contain between 0.5 mg and 5 g, preferably between 0.5 mg and 2 g, most

preferably between 0.5 mg and 1g, e.g. 50 mg, 150 mg, 250 mg, or 500 mg. Oral administration may be in one or divided doses of two, three, or four times daily.

Intravenously, the most preferred doses will range from about 0.5 to about 5 mg/kg/minute during a constant rate infusion, to achieve a plasma level concentration during the period of time of administration of between 0.1 ng/ml and 1 mg/ml.

The active drug can be administered in admixture with suitable pharmaceutical diluents, excipients or carriers (collectively referred to herein as "carrier" materials) suitably selected with respect to the intended form of administration, that is, oral tablets, capsules, elixirs, syrups and the like, and consistent with conventional pharmaceutical practices.

For instance, for oral administration in the form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic, pharmaceutically acceptable, inert carrier such as lactose, starch, sucrose, glucose, methyl cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, mannitol, sorbitol and the like; for oral administration in liquid form, the oral drug components can be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, distintegrating agents and coloring agents can also be incorporated into the mixture. Suitable binders include starch, gelatin, natural sugars such as glucose or beta-lactose, corn-sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, carboxymethylcellulose, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. Disintegrators include, without limitation, starch methyl cellulose, agar, bentonite, xanthan gum and the like.

Oral compositions of the active ingredient with enteric coatings may be prepared by mixing the active ingredient with an excipient to form a spheroid, and coating the spheroid with a thin polymer film. For example, the active ingredient is mixed with non-

water swellable microcrystalline cellulose to form a spheroid which is then coated with a film of hydroxypropyl methyl cellulose phthalate and or a plasticizer which prevents any release of the drug in the stomach. When the composition reaches the intestine, the active ingredient is released.

The compositions may also be prepared by mixing the active ingredient with a wetting agent such as fatty acid esters, lecithin, sucrose, mannitol or sorbitol and then spheronizing or granulating the mixture into microgranules. These are then coated with a microporous membrane polymer such as Eudragit ® E30D (Rohm Pharma GmbH, Weiterstadt, Germany), hydroxypropyl methyl cellulose phthalate and other wetting agents, plasticizers and the like. The formulations are enteric by nature and the active ingredient does not become bioavailable until the system reaches the intestine.

The compositions may also be prepared by mixing the active ingredient and an acid such as fumeric or tartaric acid which is compressed into a spherical tablet and coated with lacquers that are insoluble in gastric juices and soluble in intestinal juices. These lacquers include copolymers of acrylic acid and methacrylic acid esters. The acidic matrix prevents quick dissolution early and yet promotes the drugs' bioavailability further downstream in the digestive tract.

The compositions may also be prepared by coating a solid dosage form of the active ingredient with hydroxypropyl methyl cellulose phthalate or acidic succinyl and acetyl esters of hydroxypropyl methyl cellulose. Triethylcitrate is added as a plasticizer which aids in the binding of the coating material to the core pellet. The coating resists dissolution in the stomach but completely dissolves in the small intestine.

Suitable materials for providing enteric coatings include, for example, hydroxypropyl methyl cellulose phthalate, hydroxypropylmethyl cellulose acetate succinate, hydroxypropyl methyl cellulose phthalate, hydroxypropyl methyl cellulose hexahydrophthalate, shellac, cellulose acetate, cellulose acetate phthalate, polyvinyl acetate phthalate, carboxymethyl ethyl cellulose, methacrylic acid copolymers, methacrylic ester copolymers and the like.

In general, solid dosage forms comprising the active ingredient may be coated using conventional coating techniques such as conventional pan coating techniques or column spray coating techniques.

For example, coating pans, e.g. subglobular, pear shaped or hexagonal pans, which are inclined are set to rotate at an appropriate setting sufficient to allow uncoated tablets to be exposed to spray solutions of the polymer used to form the coat. The pan is heated to a sufficient temperature to allow the coat to dry soon after contact with the outside of the tablet.

Some pans have a cylindrical shape, are rotated horizontally, and have at least some regions of the walls perforated by small holes or slots. This design permits a one-way air flow through the pan. In other designs the flow of air is through the tablet bed and out through the perforated wall of the pan. In others the air flows from the perforated pan wall through the tablet bed into the central region, i.e., countercurrent to the coating spray direction. Still others permit either co- or counter-current air flow to suit particular products.

The coating is sprayed in one of several methods. One method relies entirely on hydraulic pressure to produce a spray when material is forced through a nozzle (airless spraying). In another method, atomization of the spray is assisted by turbulent jets of air. This method tends to produce a more easily controlled spray pattern and is therefore better for small-scale operations, although both are capable of giving the flat jet profile preferred for pan operation.

The thickness of coating required on the granules depends on the dissolution profile of the particular coating materials. The coating can contain a plasticizer and possibly other coating additives such as coloring agents, gloss producers, talc and/or magnesium stearate.

The active drugs can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines.

Active drug may also be delivered by the use of monoclonal antibodies as individual carriers to which the compound molecules are coupled. Active drug may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinyl-pyrrolidone, pyran copolymer, polyhydroxy-propyl-methacrylamide-phenol, polyhydroxy-ethyl-aspartamide-phenol, or polyethyleneoxide-polylysine substituted with palmitoyl residues. Furthermore, active drug may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyglycolic acid, copolymers of polylactic and polyglycolic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and cross linked or amphipathic block copolymers of hydrogels.

In ocular formulations such as eyedrops, from about 0.01-5.0% (w/v) of active ingredient can be employed, e.g., from about 0.01-2.0% (w/v) of active ingredient. Suitable eyedrop volume is, for example, 20, 30, 35, 50 or 100 ml. The objective is to administer a dose of between about 0.005-0.5 mg/kg per day to each eye, for a total dosage of between about 0.01-1.0 mg/kg/day, e.g. a dose of about 0.05 mg/kg per day to each eye, for a total dosage of about 0.1 mg/kg/day. For example, the eyedrops can be used to provide doses of 1 mg, 10 mg, or 50 mg. These dosage values are based on known and presently understood pharmacology of compounds of the invention. Dosage requirements are variable and must be individualized on the basis of the disease and the response of the patient.

Suitable eyedrop formulations are those which are isotonic and maintain sufficient contact with the eye surface to systemically deliver the active agent to the patient. Such formulations advantageously have a pH approximating neutrality and are non-irritating to the eye, e.g. they do not induce tearing and consequential flow of active agent out of the eye. Pharmaceutically acceptable carriers are, for example, water, mixtures of water and water-miscible solvents such as lower alkanols or arylalkanols, vegetable oils, polyalkylene glycols, petroleum based jelly, ethyl cellulose, hydroxy ethyl cellulose, ethyl oleate, carboxymethylcellulose, polyvinylpyrrolidone, isopropyl

myristate and other conventionally-employed non-toxic, pharmaceutically acceptable organic and inorganic carriers. The pharmaceutical preparation may also contain non-toxic auxiliary substances such as emulsifying, preserving, wetting agents, bodying agents and the like, as for example, polyethylene glycols 200, 300, 400 and 600, carbowaxes 1000, 1500, 4000, 6000 and 10000, antibacterial compounds, phenylmercuric salts known to have cold sterilizing properties and which are non-injurious in use, thimerosal, methyl and propyl paraben, benzyl alcohol, phenyl ethanol, buffering ingredients such as sodium chloride, sodium borate, sodium acetates, gluconate buffers, and other conventional ingredients such as sorbitan monolaurate, triethanolamine, oleate, polyoxyethylene sorbitan monopalmitylate, dioctyl sodium sulfosuccinate, monothioglycerol, thiosorbitol, ethylenediamine tetraacetic acid, and the like. Additionally, suitable ophthalmic vehicles can be used as carrier media for the present purpose including conventional phosphate buffer vehicle systems, isotonic boric acid vehicles, isotonic sodium chloride vehicles, isotonic sodium borate vehicles and the like.

In the procedure for making eyedrops, formulations are rendered sterile by appropriate means, such as starting the preparation procedure with sterile components and proceeding under sterile conditions, irradiating or autoclaving the finished formulation, and the like. Suitable anti microbial agents are also useful for maintaining sterility of the eyedrop.

The ocular preparation may also be a solid insert such as one which, after dispensing the compound, remains essentially intact, or a bioerodible insert that is soluble in lacrimal fluids, or otherwise disintegrates. For example, one may use a solid water soluble polymer as the carrier for the compound. The polymer used to form the insert may be any water soluble non-toxic polymer, for example, cellulose derivatives such as methylcellulose, sodium carboxymethyl cellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethyl cellulose, acrylates such as polyacrylic acid salts, ethylacrylates, polyacrylamides, natural products such as gelatin, alginates, pectins, tragacanth, karaya, chondrus, agar, acacia, starch derivatives such as

starch acetate, hydroxyethyl starch ethers, hydroxypropyl starch, as well as other synthetic derivatives such as polyvinyl alcohol, polyvinyl pyrrolidone, polyvinyl methyl ether, polyethylene oxide, neutralized carbopol, gellan gum and xanthan gum, and mixtures of said polymers.

The ocular preparation may also be an ointment which is compounded, for example, by mixing finely milled powdered ingredients with a small amount of white petrolatum and levigating or otherwise mixing until a uniform distribution is achieved. The balance of white petrolatum is added by geometric addition until the desired dosage form is made.

Thrombin inhibitors

Suitable thrombin inhibitors include those which inhibit thrombosis, including but not limited to those described in U.S. Patent 5,536,708, U.S. Patent 5,510,369, WO 96/31504, WO 96/32110 and WO 97/15190. Such compounds are useful for treating or preventing venous thromboembolism (e.g. obstruction or occlusion of a vein by a detached thrombus; obstruction or occlusion of a lung artery by a detached thrombus), cardiogenic thromboembolism (e.g. obstruction or occlusion of the heart by a detached thrombus), arterial thrombosis (e.g. formation of a thrombus within an artery that may cause infarction of tissue supplied by the artery), atherosclerosis (e.g. arteriosclerosis characterized by irregularly distributed lipid deposits) in mammals, and for lowering the propensity of devices that come into contact with blood to clot blood.

Examples of venous thromboembolism which may be treated or prevented with thrombin inhibitors include obstruction of a vein, obstruction of a lung artery (pulmonary embolism), deep vein thrombosis, thrombosis associated with cancer and cancer chemotherapy, thrombosis inherited with thrombophilic diseases such as Protein C deficiency, Protein S deficiency, antithrombin III deficiency, and Factor V Leiden, and thrombosis resulting from acquired thrombophilic disorders such as systemic lupus erythematosus (inflammatory connective tissue disease). Also with regard to venous

thromboembolism, thrombin inhibitors are useful for maintaining patency of indwelling catheters.

Examples of cardiogenic thromboembolism which may be treated or prevented with thrombin inhibitors include thromboembolic stroke (detached thrombus causing neurological affliction related to impaired cerebral blood supply), cardiogenic thromboembolism associated with atrial fibrillation (rapid, irregular twitching of upper heart chamber muscular fibrils), cardiogenic thromboembolism associated with prosthetic heart valves such as mechanical heart valves, and cardiogenic thromboembolism associated with heart disease.

Examples of arterial thrombosis include unstable angina (severe constrictive pain in chest of coronary origin), myocardial infarction (heart muscle cell death resulting from insufficient blood supply), ischemic heart disease (local anemia due to obstruction (such as by arterial narrowing) of blood supply), reocclusion during or after percutaneous transluminal coronary angioplasty, restenosis after percutaneous transluminal coronary angioplasty, occlusion of coronary artery bypass grafts, and occlusive cerebrovascular disease. Also with regard to arterial thrombosis, thrombin inhibitors are useful for maintaining patency in arteriovenous cannulas.

Examples of atherosclerosis include arteriosclerosis.

Examples of devices that come into contact with blood include vascular grafts, stents, orthopedic prosthesis, cardiac prosthesis, and extracorporeal circulation systems

Tissue factor pathway inhibitor

These inhibitors block the trauma response which ordinarily results in the release from blood vessels of lipoprotein tissue factor. Tissue factor serves as a stimulatory protein, which, together with factor VIIa, a serine protease, catalyzes the activation of Factor X.

Thrombin receptor antagonists

These compounds block the binding to thrombin receptors of thrombin, which is a proteolytic enzyme which converts fibrinogen into fibrin.

Factor Xa inhibitors

Factor Xa inhibitors are useful for the treatment of arterial and venous thrombotic occlusive disorders, inflammation, cancer, and neurodegenerative diseases. Suitable factor Xa inhibitors include, but are not limited to, those described in U.S. Patent 5,633,381, U.S. Patent 5,612,378, U.S. Patent 5,612,363, U.S. Patent 5,612,353, and WO 97/23212, the contents of each of which are hereby incorporated by reference.

Low molecular weight heparin

Low molecular weight heparin is also suitable for use as the antithrombotic agent. Low molecular weight heparin is a mixture of low molecular weight sulfated glycoaminoglycans obtained either by fractionation or chemical hydrolysis of standard heparin with nitrous oxide. The molecular weights of the constituent molecules range from about 2000 to 5000. These molecules form binary complexes with antithrombin III, thus inhibiting only Factor Xa and not thrombin. These molecules bound to antithrombin III react only slightly with platelets. Consequently, deep-vein thrombosis can be prevented or retarded without as much risk of hemorrhage as with standard heparin. Suitable low molecular weight heparins include, but are not limited to, those described in U.S. Patent 5,614,494, U.S. Patent 5,534,619, U.S. Patent 5,576,304, and U.S. Patent 5,474,987, the contents of each of which are hereby incorporated by reference.

Angiogenesis promoters

Suitable angiogenesis promoters include, but are not limited to, vascular endothelial growth factors which are members of the endothelial motigen family (WO 97/09427; Joukov et al., The EMBO Journal, vol. 15 no. 2 pp. 290-298 (1996)) such as VEGF 189 (U.S. Patent 5,240,848), VEGF 165 (U.S. Patent 5,219,739; U.S. Patent 5,332,671), VEGF 145 (Charnock-Jones et al (1993, *Biol. Reproduction* 48: 1120-1128) and Sharkey et al (1993, *J. Reprod. Fertility* 99, 609-615); Poltorak et al. (1997, *J. Biol. Chem.* 272, 7151-7158)), VEGF 121 (U.S. Patent 5,219,739; U.S. Patent 5,194,596), or VEGF 120 (U.S. Patent 5,219,739), rat VEGF sequences, VEGF-B (Grimmond et al., 1996, *Genome Research* 6: 124-

131; Olofsson et al., 1996, *Proc. Natl. Acad. Sci. USA* 93: 2576-2581), VEGF-C (Joukov et al., 1996, *EMBO J.* 15: 290-298; see also PCT International application WO 96/39515), VEGF-D (Yamada, et al., 1997, *Genomics* 42:483-488), human placenta growth factor (PlGF)(Bayne and Thomas, EP Publication 0 506 477 [30 Sept 1992]; Maglione, et al., 1993, *Oncogene* 8: 925-931; Hauser and Weich, 1993, *Growth Factors* 9: 259-268), a fibroblast growth factor which is a vascular endothelial cell mitogen such as acidic fibroblast growth factor (aFGF)(U.S. Patent 4,444,760; U.S. Patent 5,312,911; U.S. Patent 5,401,832; U.S. Patent 5,348,941; U.S. Patent 5,409,897; U.S. Patent 5,439,818; U.S. Patent 5,514,566), modified aFGF such as aFGF Ser 117) or basic fibroblast growth factor (bFGF)(U.S. Patent 5,604,293; U.S. Patent 5,217,954; U.S. Patent 5,439,818; U.S. Patent 5,223,483; U.S. Patent 5,514,566), a platelet derived growth factor (PDGF AA, AB or BB), an insulin-like growth factor (U.S. Patent 4,876,242) (e.g. IGF-I (U.S. Patent 5,565,428; U.S. Patent 5,583,109; Jabri et al., Diabetes, vol. 43 pp. 369-374 (1994); Rinderknecht et al., Proc. Natl. Acad. Sci. USA, vol. 73 no. 7 pp. 2365-2369 (1976)) or IGF-II (Rinderknecht et al., Proc. Natl. Acad. Sci. USA, vol. 73 no. 7 pp. 2365-2369 (1976)), or a hepatocyte growth factor (HGF)(U.S. Patent 5,654,404; U.S. Patent 5,606,029; U.S. Patent 5,589,451; U.S. Patent 5,587,309; U.S. Patent 5,580,963; U.S. Patent 5,316,921; U.S. Patent 5,571,509).

Vascular endothelial growth factors and fibroblast growth factors are particularly suited for use in the present invention since they induce plasminogen activators and may augment the effect of the antithrombotic used in the method. Also, fibroblast growth factors are particularly suited for this method since they decrease expression of the plasminogen activator inhibitor PAI-I (which can lead to increased clot lysis). Vascular endothelial growth factors are also particularly suited for combination therapy with antithrombotics since these growth factors promote more rapid endothelialization at the site of plaque rupture, and provide an antirestenosis effect. Thus, the acute restenosis benefit of antithrombotics, along with the chronic therapeutic benefit of these growth factors, make the antirestenosis benefit of the combination particularly effective and desirable.

Administration of the angiogenesis promoters may be by any suitable means known to persons skilled in the art, e.g. via bolus injection, continuous intravenous administration, coated stent implantation (intramuscular injection on a balloon, intraarterial injection on a balloon) and gene transfer, which provides either systemic or localized delivery of the angiogenesis promoter at the ischemic tissue. Genetic delivery may be obtained using modified vector or adenoviruses (Adenovirus might be given systemically, expressed in liver and spleen and circulating VEGF then perform like i.v. injected protein) that enter via receptor-mediated gene transfer modification of the vector or virus for cell receptor specificity which leads to endocytosis, e.g., pinocytosis or phagocytosis, whereby the angiogenesis promoter is produced and then taken into a cell by invagination of the plasma membrane, which it breaks off as a boundary membrane of the part engulfed.

Acidic fibroblast growth factor and basic fibroblast growth factor genes are especially efficient as gene therapeutics if fused to 5' DNA encoding an artificial secretory leader peptide sequence since they are devoid of such an endogenous sequence (i.e., secretory leaders are not required).

For treatment of ischemic tissue, the angiogenesis promoter will be formulated and dosed in a fashion consistent with good medical practice taking into account the condition of the individual patient, the site of delivery of the promoter, the method of administration, and other factors known to practitioners.

The angiogenesis promoter may be administered by localized gene transfer delivery. Gene transfer involves transfer of vectors to a host cell, such as a eukaryotic host cell. Cells may be contacted with the vectors by any means by which the vectors will be introduced into the cell. The viral vectors can be introduced by infection using the natural capability of the virus to enter cells (e.g., the capability of adenovirus to enter cells via receptor-mediated endocytosis). However, the viral and plasmid vectors can be introduced by any other suitable means, e.g., transfection, calcium phosphate-mediated transformation, microinjection, electroporation, osmotic shock, and the like. The method contemplates vector transfer *in vivo* by the methods set forth in

WO 97/09439, e.g., liposome/DNA/adenoviruses (synthetic like virus particles), or viruses modified for tropism in response to positive or negative stimuli, or by any other standard method.

The method provides recombinase encoded by an integrated or episomal vector, or the recombinase in the form of protein. The vector can be integrated into the host chromosome or can be episomal, wherein an extrachromosomal element replicates and functions stably when physically separated from the host chromosome. Introduction of protein can be effected by any means appropriate for protein introduction to a cell, e.g., microinjection and adenoviral-mediated uptake for *in vitro* delivery, and injection or infusion or further means as described herein for *in vivo* delivery, as well as by other standard means of introduction known by those skilled in the art.

When practiced *in vivo*, suitable organs or tissues or component cells targeted for vector or protein delivery include organs/tissues/cells of the circulatory system (i.e., heart, blood vessels or blood).

A composition for delivery of the therapeutic gene (i.e., a composition comprising the vectors and/or recombinases) can be made into a pharmaceutical composition with appropriate pharmaceutically acceptable carriers or diluents, and where appropriate, can be formulated into preparations in solid, semi-solid, liquid or gaseous forms such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, and aerosols, in the usual ways for their respective route of administration. Means known in the art can be utilized to prevent release and absorption of the composition until it reaches the target organ, tissue, or cell, or to ensure timed-release of the composition. A pharmaceutically acceptable form should be employed which does not ineffectuate the composition. In pharmaceutical dosage forms, the compositions can be used alone or in appropriate association, as well as in combination with, other pharmaceutically active compounds.

Accordingly, the pharmaceutical composition can be delivered via various routes and to various sites in an animal body to achieve a particular effect. Local or systemic delivery can be

accomplished by administration comprising application or instillation of the formulation into body cavities, inhalation or insufflation or an aerosol, or by parenteral introduction, comprising intramuscular, intravenous, peritoneal, subcutaneous, intraderma, as well as topical administration.

The composition can be provided in unit dosage form wherein each dosage unit, e.g., a teaspoonful, tablet, solution, or suppository, contains a predetermined amount of the composition, alone or in appropriate combination with other active agents. The term "unit dosage form" as used herein refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of the composition of the present invention, alone or in combination with other active agents, calculated in an amount sufficient to produce the desired effect, in association with a pharmaceutically acceptable diluent, carrier, or vehicle, where appropriate. The specifications for the novel unit dosage forms depend on the particular effect to be achieved and the particular pharmacodynamics associated with the pharmaceutical composition in the particular host.

Accordingly, the compositions are useful in a method of obtaining stable gene expression in a host, or modulating gene expression in a host. The composition is administered using any of the aforementioned routes of administration or alternative routes known to those skilled in the art and appropriate for a particular application. The "effective amount" of the composition is such as to produce the desired effect in a host which can be monitored using several end-points known to those skilled in the art. For example, one desired effect might comprise effective nucleic acid transfer to a host cell. Such transfer could be monitored in terms of an angiogenesis promoting therapeutic effect, (such as a stress test, walking test, angiography etc.) or by further evidence of the transferred gene or coding sequence or its expression within the host (e.g., using the polymerase chain reaction, Northern or Southern hybridizations, or transcription assays to detect the nucleic acid in host cells, or using immunoblot analysis, antibody-mediated detection, or particularized assays to detect protein or polypeptide

encoded by the transferred nucleic acid, or impacted in level or function due to such transfer).

These methods described are by no means all-inclusive, and further methods to suit the specific application will be apparent to the ordinary skilled artisan. Moreover, the effective amount of the composition can be further approximated through appropriate assay of the recombination reaction, as previously described.

Generally, to ensure effective transfer of the vectors of the present invention, it is preferable that about 1 to about 5,000 copies of the vector be employed per cell to be contacted, based on an approximate number of cells to be contacted in view of the given route of administration, and even more preferable that about 1 to about 300 pfu enter each cell. However, this is merely a general guideline which by no means precludes use of a higher or lower amount of a component, as might be warranted in a particular application, either *in vitro* or *in vivo*. For example, the actual dose and schedule can vary depending on whether the composition is administered in combination with other pharmaceutical compositions, or depending on inter individual differences in pharmacokinetics, drug disposition, and metabolism. One skilled in the art easily can make any necessary adjustments in accordance with the necessities of the particular situation.

WO 97/09439, which describes a suitable means for delivering an angiogenesis promoter which involves localized delivery of a therapeutic gene, is incorporated by reference.

WO 97/09439 describes two methods to effect site-specific recombination in a cell. Both recombination methods involve contacting a cell with a vector comprising first and second recombining sites such that the vector is internalized by the cell, and then providing the cell with a site-specific recombinase that effects recombination between the first and second recombining sites of the vector. The first site-specific recombination method involves the use of a vector (i.e., a "parallel recombination vector") comprising first and second recombining sites located in a parallel orientation. The second site-specific recombination method involves the use of a vector (i.e., an "antiparallel recombination

vector") comprising the first and second recombining sites located in an antiparallel (or opposite) orientation.

The parallel recombination method is capable of effecting site-specific recombination in a cell such that the recombination event generates an episome comprising an origin of replication capable of functioning in mammalian cells, which can replicate autonomously of the host genome and can be employed to impart stable maintenance in host cells upon vectors carrying one or more passenger genes. The parallel recombination method is useful in that it can be employed to stabilize vectors which typically do not integrate into the host cell genome (e.g., Ad or HSV vectors) by imparting to such vectors an ability to replicate via a "lysogenic-like" pathway. Thus, the method can be employed to obtain stable gene expression by stabilizing vectors carrying one or more passenger genes.

The parallel recombination method also can be employed to effect site-specific recombination in a cell such that the recombination event generates a so-called "miniplasmid", which does not comprise an origin of replication that is capable of functioning in mammalian cells, and which is not capable of replicating autonomously of the host genome. Notably, the second recombination product generated by the parallel recombination event (i.e., the product other than the episome or miniplasmid, which itself comprises either a linear or a closed-circular structure depending on whether the original substrate for the recombination reaction is, respectively, either linear or closed-circular) also may be functional in the cell. Such a miniplasmid, as further described herein, can prove of use for the short-term delivery of proteins (e.g., Rep protein) to cells.

Alternatively, it also is possible that the vector employed for site-specific recombination comprises more than one origin of replication prior to the recombination event. Vectors that comprise more than one replication origin are known in the art.

As described further herein, both the parallel and antiparallel recombination methods can be employed to either up- or down-regulate transcription of a coding sequence, or to simultaneously up-regulate transcription of one coding sequence and down-regulate

transcription of another, through the recombination event. The antiparallel recombination method differs from the parallel recombination method in that it does not involve the formation of an episome or miniplasmid.

The recombination methods according to the invention are in a cell, which preferably is a eukaryotic cell. A eukaryotic cell is a cell which posses a true nucleus surrounded by a nuclear membrane. Preferably the eukaryotic cell is of a multicellular species (e.g., as opposed to a unicellular yeast cell), and even more preferably is a mammalian (optimally human) cell. However, the methods also can be effectively carried out using a wide variety of different cell types such as avian and fish cells, and mammalian cells including but not limited to rodent, ape, chimpanzee, feline, canine, ungulate (such as ruminant or swine), as well as human cells. Moreover, if vector transfer to a particular cell type is limited due, for instance, to a lack of receptors for a particular virus such as adenovirus, transfer can be increased using methods employed, for example, to carry human adenovirus into blood or other cell types. For instance, the virus can be coupled to a DNA-polylysine complex containing a ligand (e.g., transferrin) for mammalian cells (Wagner et al., Proc. Natl. Acad. Sci. USA, 89, 6099-6103 (1992)), or by using other similar methods which are known to those skilled in the art. Transfer can also be increased by modifying the adenovirus coat, or modifying the vector to increase efficacy of endosome escape/transfer to the nuclear membrane, of mRNA synthesis with splicing, and of transcription/translation using a modified polyadenylate tail.

Any suitable vector can be utilized in the methods. Thus, the vector utilized can encompass any vector, linear or circular, that is appropriate for introduction of nucleic acids into eukaryotic cells, and is capable of functioning as a vector as that term is understood by those of ordinary skill in the art, so long as the vector is predominantly comprised of double-stranded DNA during some phase of its existence. Preferably, the resultant vector is compatible with the host cell, i.e., is capable of transcribing the nucleic acid sequences subcloned in the vector, and if desired, also is capable of translating the nascent mRNA.

The vector optimally is of viral origin, and can contain one or more heterologous or recombinant sequences, e.g., a coding sequence, a passenger gene, promoter, or the like. Preferably the vector contains the minimal sequences required for packaging and delivery to the cell. The vector desirably is comprised, in part, of a virus that is either enveloped or nonenveloped. For instance, preferably a vector is a nonenveloped virus from the family *Hepadnaviridae*, *Parvoviridae*, *Papovaviridae*, or *Adenoviridae*. A preferred nonenveloped virus according to the invention is a virus of the family *Hepadnaviridae*, especially of the genus *Hepadnavirus*. A virus of the family *Parvoviridae* desirable is of the genus Parvovirus (e.g., parvoviruses of mammals and birds) or *Dependovirus* (e.g., adeno-associated viruses (AAVs)). A virus of the family *Papovaviridae* preferably is of the subfamily *Papillomavirinae* (e.g., the papillomaviruses including, but not limited to, human papillomaviruses (HPV) 1-48 and bovine papillomaviruses (BPV)) or the subfamily *Polyomavirinae* (e.g., the polyomaviruses including, but not limited to, JC, SV40, BK virus, and mouse polyomavirus). A virus of the family *Adenoviridae* desirably is of the genus *Mastadenovirus* (e.g., mammalian adenoviruses) or *Aviadenovirus* (e.g., avian adenoviruses). Similarly, a vector can be an enveloped virus from the family *Herpesviridae*, or can be a Sindbis virus. A preferred envelope virus according to the invention is a virus of the family *Herpesviridae*, especially of the subfamily *Alphaherpesvirinae* (e.g., the herpes simplex-like family viruses), genus *Simplexvirus* (e.g., herpes simplex-like viruses), genus *Varicellavirus* (e.g., varicella and pseudorabies-like viruses), subfamily *Betaherpesvirinae* (e.g., the cytomegaloviruses), genus *Cytomegalovirus* (e.g., the human cytomegaloviruses), subfamily *Gammaherpesvirinae* (e.g., the lymphocyte-associated viruses), or genus *Lymphocryptovirus* (e.g., EB-like viruses). In particular, preferably the virus component of the vector is selected from the group consisting of Ad, herpes simplex virus types I and II (HSV), EBV, vaccinia virus, papilloma virus (e.g., either human (HPV) or bovine (BPV)), JC, simian virus 40 (SV40), polyomavirus (e.g., either human or mouse), hepatitis virus B, and cytomegalovirus (CMV). Moreover, for practice of the parallel recombination method wherein an

episome desirably is formed, preferably, the vector is comprised, in part, of vectors derived from viruses which do not form proviruses as part of their replicative cycle.

The angiogenesis promoter may also be administered by coating a stent with the angiogenesis promoter and inserting the stent at the site of ischemic tissue damage in accordance with ordinary coronary stent procedures. Such stents are wrapped in polymer films capable of carrying and releasing the angiogenesis promoter. Preparation of such coated stents is described in European Publication 716836, the contents of which are hereby incorporated by reference.

Alternatively, the angiogenesis promoter may be administered as either a solution, spray, gel, cream, ointment, or dry powder directly to the site of injury. Slow-release devices directing the angiogenesis promoter to the injured site may also be used. In direct applications, such as injection, the angiogenesis promoter will be applied at a concentration ranging from about 50 to 1,000 mg/mL, either in a single application, or in dosing regimens that are daily or every few days for a period of one week to several weeks.

The angiogenesis promoter can be used as a post-operative wound healing agent in balloon angioplasty, a procedure in which vascular endothelial cells are removed or damaged, together with compression of atherosclerotic plaques. It can also be used in percutaneous transluminal coronary revascularization, directional coronary angioplasty, percutaneous rotational coronary atherectomy, excimer laser coronary atherectomy, etc. The angiogenesis promoter can be applied to inner vascular surfaces by systemic or local intravenous application either as intravenous bolus injection or infusions. If desired, the angiogenesis promoter can be administered over time using a micro metering pump. Suitable compositions for intravenous administration comprise the angiogenesis promoter in an amount effective to promote endothelial cell growth and a parenteral carrier material. The angiogenesis promoter can be administered using injections of 3 to 10 mL per patient once or in dosing regimens that allow for multiple applications. Any of the known parenteral carrier vehicles can be used, such as normal saline or 5-10% dextrose.

The angiogenesis promoter can also be employed to repair vascular damage following myocardial infarction and to circumvent the need for coronary bypass surgery by stimulating growth of a collateral circulation. The angiogenesis promoter is administered intravenously for this purpose, either in individual injections or by micro metering pump over a period of time as described above or by direct infusion or injection to the site of damaged cardial muscle.

Therapeutic formulations of angiogenesis promoter are prepared for storage by mixing angiogenesis promoter having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences, 16th edition, Osol, A., Ed., [1980]), in the form of lyophilized cake or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidione; amino acids such as glycine, glutamine, asparagine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counter-ions such as sodium; and/or non-ionic surfactants such as Tween, pluronics or polyethylene glycol (PEG).

The angiogenesis promoter also may be entrapped in micro capsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-micro capsules and poly-[methylmethacylate] micro capsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, *supra*.

Angiogenesis promoter to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and

reconstitution. Angiogenesis promoter ordinarily will be stored in lyophilized form or in solution.

Therapeutic angiogenesis promoter compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The route of angiogenesis promoter administration is in accord with known methods, e.g., those routes set forth above for specific indications, as well as the general routes of injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, or intralesional means, or sustained release systems as noted below. Angiogenesis promoter is administered continuously by infusion or by bolus injection. Generally, for ischemic tissue repair, one should formulate and dose the angiogenesis promoter for site-specific delivery.

Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the protein, which matrices are in the form of shaped articles, e.g., films, or micro capsules. Examples of sustained-release matrices include polyesters, hydrogels [e.g., poly(2-hydroxyethyl-methacrylate) as described by Langer *et al.*, J. Biomed. Mater. Res., 15:167-277 (1981) and Langer, Chem. Tech., 12:98-105 (1982) or poly(vinylalcohol)], polylactides (U.S. Patent No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, Biopolymers, 22:547-556 [1983]), non-degradable ethylene-vinyl acetate (Langer *et al.*, *supra*), degradable lactic acid-glycolic acid copolymers such as the Lupron Depot™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutryric acid (EP 133 988).

While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated proteins remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational

strategies can be devised for protein stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

Sustained-release angiogenesis promoter compositions also include liposomally entrapped angiogenesis promoter. Liposomes containing angiogenesis promoter are prepared by methods known *per se:* DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. USA, 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci. USA, 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese patent application 83-118008; U.S. Patent Nos. 4,485,045 and 5,544,545; and EP 102,324. Ordinarily the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol.% cholesterol, the selected proportion being adjusted for the optimal angiogenesis promoter therapy.

When applied topically (i.e., directly to the damaged tissue), the angiogenesis promoter is suitably combined with other ingredients, such as carriers and/or adjuvants. There are no limitations on the nature of such other ingredients, except that they must be pharmaceutically acceptable and efficacious for their intended administration, and cannot degrade the activity of the active ingredients of the composition. Examples of suitable vehicles include ointments, creams, gels, or suspensions, with or without purified collagen. The compositions also may be impregnated into transdermal patches, plasters, and bandages, preferably in liquid or semi-liquid form.

For obtaining a gel formulation, the angiogenesis promoter formulated in a liquid composition may be mixed with an effective amount of a water-soluble polysaccharide or synthetic polymer such as PEG to form a gel of the proper viscosity to be applied topically. The polysaccharide that may be used includes, for example, cellulose derivatives such as etherified cellulose derivatives, including alkyl celluloses, hydroxyalkyl celluloses, and alkylhdroxyalkyl celluloses, for

example, methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxypropyl methylcellulose, and hydroxypropyl cellulose; starch and fractionated starch; agar, alginic acid and alginates; gum arabic; pullullan; agarose; carrageenan; dextrans; dextrins; fructans; insulin; mannans; xylans; arabinans; chitosans; glycogens; glucans; and synthetic biopolymers; as well as gums such as xanthan gum; guar gum; locust bean gum; gum arabic; tragacanth gum; and karaya gum; and derivatives and mixtures thereof. The preferred gelling agent herein is one that is inert to biological systems, nontoxic, simple to prepare, and not too runny or viscous, and will not destabilize the angiogenesis promoter held within it.

Preferably the polysaccharide is an etherified cellulose derivative, more preferably one that is well defined, purified, and listed in USP, e.g., methylcellulose and the hydroxyalkyl cellulose derivatives, such as hydroxypropyl cellulose, hydroxyethyl cellulose, and hydroxypropyl methylcellulose. Most preferred herein is methylcellulose.

The polyethylene glycol useful for gelling is typically a mixture of low and high molecular weight PEGs to obtain the proper viscosity. For example, a mixture of PEG of molecular weight 400-600 with one of molecular weight 1500 would be effective for this purpose when mixed in the proper ratio to obtain a paste.

The term "water soluble" as applied to the polysaccharides and PEGs is meant to include colloidal solutions and dispersions. In general, the solubility of the cellulose derivatives is determined by the degree of substitution of ether groups, and the stabilizing derivatives useful herein should have a sufficient quantity of such ether groups per anhydroglucose unit in the cellulose chain to render the derivatives water soluble. A degree of ether substitution of at least 0.35 ether groups per anhydroglucose unit is generally sufficient. Additionally, the cellulose derivatives may be in the form of alkali metal salts, for example, the Li, Na, K, or Cs salts.

If methylcellulose is employed in the gel, preferably it comprises about 2-5%, more preferably about 3%, of the gel and the

angiogenesis promoter is present in an amount of about 300-1000 mg per ml of gel.

An effective amount of angiogenesis promoter to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. Typically, the clinician will administer the angiogenesis promoter until a dosage is reached that achieves the desired effect. A typical daily dosage for systemic treatment might range from about 1 mg/kg to up to 10 mg/kg or more, depending on the factors mentioned above. As an alternative general proposition, the angiogenesis promoter is formulated and delivered to the target site or tissue at a dosage capable of establishing in the tissue an angiogenesis promoter level greater than about 0.1 ng/cc up to a maximum dose that is efficacious but not unduly toxic. This intra-tissue concentration should be maintained if possible by continuous infusion, sustained release, topical application, or injection at empirically determined frequencies. The progress of this therapy is easily monitored by conventional assays.

It is within the scope hereof to conduct angiogenesis promoter therapy with more than one angiogenesis promoter for enhancing the activity of any of the growth factors in promoting cell proliferation and repair. It is not necessary that such co-treatment drugs be included *per se* in the compositions of this invention, although this will be convenient where such drugs are proteinaceous. Such admixtures are suitably administered in the same manner and for the same purposes as when only one angiogenesis promoter is used. The useful molar ratio of the angiogenesis promoters is typically 1:0.1-10.

Therapeutic Treatment

The method of the invention is useful for treating patients where inhibition of human or mammalian acute coronary ischemic syndrome is desired. The method is useful in surgery on peripheral arteries (arterial grafts, carotid endaterectomy) and also prevention of

stroke by PTCA of coronary artery, and vertebral artery stenosis, and in cardiovascular surgery where manipulation of arteries and organs, and/or the interaction of platelets with artificial surfaces, leads to platelet aggregation and potential formation of thrombi and thromboemboli. The method is also useful for treating general peripheral arterial disease, for treating amputation patients, and for treating ischemic ulcer patients. Compositions and methods of the invention may be used to prevent the formation of thrombi and thromboemboli.

The present invention is demonstrated in treatment of patients with acute coronary ischemic syndromes who are undergoing early coronary revascularization with percutaneous coronary angioplasty or atherectomy, or in the setting of minimally invasive bypass graft surgery or bypass surgery. Acute coronary ischemic syndrome is associated with death and nonfatal myocardial infarction, and subsequent follow-up procedures such as coronary artery bypass grafting, repeat percutaneous intervention for acute ischemia, and insertion of a coronary endovascular stent. Because of unstable plaque with thrombus, percutaneous revascularization procedures in these patients carry with them considerable higher morbidity than procedures performed in patients with stable coronary disease.

Patients receive a GP IIb/IIIa receptor antagonist via ordinary means such as bolus injection, continuous intravenous or oral administration, and optionally additionally heparin (a standard PTCA regimen, weight adjusted in lighter patients), aspirin or other agent which is suitably administered with a GP IIb/IIIa receptor antagonist . When heparin is used, it may be discontinued after completion of the procedure and sheaths removed when the heparin-effect has dissipated. GP IIb/IIIa receptor antagonist therapy is continued thereafter, e.g. for 24 hours.

Angiogenesis promoter is introduced to provide therapeutic benefit of angiogenesis promotion at the site of ischemic tissue damage, and also to minimize or reduce the severity and incidence of clinical restenosis. For example, gene therapy is effected by transferring the gene corresponding to the angiogenesis promoter to damaged tissue.

Alternatively, angiogenesis promoter may be delivered by other means, e.g. implantation of angiogenesis promoter coated stent or multiple dose administration of the angiogensis promoter.

Coronary ischemia and peripheral ischemia concern formation of thrombi on the heart or arterial wall. Thrombi precipitate acute events. Once formed, the thrombus often persists and becomes incorporated into the wall, contributing to the growth of the plaque and perpetuation of the ischemia.

EXAMPLE 1

GP IIb/IIIa antagonist/vascular endothelial growth factor treatment

Patients with acute coronary ischemic syndromes receive coronary revascularization with angioplasty. Aspirin is administered in a dose of 325 mg at least two hours before angioplasty, and daily thereafter. Heparin is given intravenously in an initial bolus dose of 10,000 to 12,000 units followed by incremental bolus doses of up to 3000 units at 15-minute intervals, but no more than 20,000 units is given during the procedure. The goal is to keep the activated clotting time between 300 and 350 seconds during the operation. Heparin is continued by constant infusion for at least 12 hours to maintain the activated partial-thromboplastin time at 1.5 to 2.5 times the control value. Aspirin is required at discharge in a dose of 325 mg per day.

Patients receive oral administration of 15 mg fibrinogen receptor gp IIb/IIIa antagonist (R)-methyl-3-[[[3-[4-(aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-N-(butoxycarbonyl)-L-alanine monoacetate each day.

Vascular endothelial growth factor 145 therapy is initiated by transferring the growth factor gene to the damaged tissue. Patient response will include reduced incidences of clot formation and enhanced vascular growth.

EXAMPLE 2

GP IIb/IIIa antagonist tablet preparation

Tablets containing 15 mg of the fibrinogen receptor gp IIb/IIIa antagonist (R)-methyl-3-[[[3-[4-(aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-N-(butoxycarbonyl)-L-alanine monoacetate (compound 2-1) used in the procedure of Example 1 are prepared as illustrated below:

Ingredient	mg
2-1	15.0
Microcrystalline cellulose	400.0
Modified food corn starch	17.0
Magnesium stearate	3.0

Compound 2-1, cellulose, and a portion of the corn starch are mixed and granulated to 10% corn starch paste. The resulting granulation is sieved, dried and blended with the remainder of the corn starch and the magnesium stearate. The resulting granulation is then compressed into tablets.

EXAMPLE 3

Example 1 is repeated, except gp IIb/IIIa antagonist (R)-methyl-3-[[[3-[4-(aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-N-(butoxycarbonyl)-L-alanine monoacetate and vascular endothelial growth factor 145 therapy is initiated by implanting a stent coated with both the antagonist and the growth factor to inhibit aggregation and enhance vascular growth at the site of tissue damage. Patient response will include reduced incidences of clot formation and enhanced vascular growth.

EXAMPLE 4

Example 1 is repeated, except vascular endothelial growth factor 145 therapy is initiated by implanting a stent coated with the growth factor to enhance vascular growth at the site of tissue damage. Patient response will include reduced incidences of clot formation and enhanced vascular growth.

EXAMPLE 5

Example 1 is repeated, except vascular endothelial growth factor 145 therapy is initiated by multiple bolus dose administration of growth factor to enhance vascular growth at the site of tissue damage. Patient response will include reduced incidences of clot formation and enhanced vascular growth.

EXAMPLES 6-10

Examples 1-5 are repeated except that acidic fibroblast growth factor is used as the angiogensesis promoter instead of vascular endothelial growth factor 145.

WHAT IS CLAIMED IS:

1. A method for treating a patient having coronary ischemic syndrome by administering to the patient an effective amount of an antithrombotic agent and an effective amount of an angiogenesis promoter.

2. A method of Claim 1 wherein the antithrombotic agent is selected from the group consisting of a glycoprotein IIb/IIIa antagonist, a thrombin inhibitor, a factor Xa inhibitor, a tissue factor pathway inhibitor, a thrombin receptor antagonist, or a low molecular weight heparin.

3. A method of Claim 1, wherein the angiogenesis promoter is administered by means selected from the group consisting of bolus injection, continuous intravenous administration, coated stent implantation and gene transfer, which means provides localized delivery of the angiogenesis promoter to the ischemic tissue.

4. A method of Claim 3, wherein the angiogenesis promoter is administered by means of gene transfer, which means provides localized delivery of the angiogenesis promoter to the ischemic tissue.

5. A method of Claim 1, wherein the angiogenesis promoter is a vascular endothelial growth factor, hepatocyte growth factor or a fibroblast growth factor.

6. A method of Claim 2 wherein the antithrombotic agent is a glycoprotein IIb/IIIa antagonist.

7. A method of Claim 5 wherein the vascular endothelial growth factor is selected from the group consisting of VEGF-A, isoforms VEGF 206, VEGF 189, VEGF 165, VEGF 145, VEGF 121, VEGF-B, VEGF-C, VEGF-D, VEGF A/VEGF B heterodimer, VEGF

A/VEGF C heterodimer, VEGF A/VEGF D heterodimer, VEGF A/PlGF heterodimer.

8. A method of claim 5 wherein the fibroblast growth factor is selected from the group consisting of acidic fibroblast growth factor, basic fibroblast growth factor, FGF-3, FGF-4, FGF-5, FGF-6, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14 and FGF-15.

9. A method of claim 2 wherein the glycoprotein IIb/IIIa receptor antagonist is selected from the group consisting of

Mpr-(Acetimidyl-Lys)-Gly-Asp-Trp-Phe-Cys-NH_2,

Mpr-(Acetimidyl-Lys)-Gly-Asp-Trp-Phe-Pen-NH_2,

Mpr-(Phenylimidyl-Lys)-Gly-Asp-Trp-Phe-Pen-NH_2,

Mpr-(Phenylimidyl-Lys)-Gly-Asp-Trp-Phe-Cys-NH_2,

N-Methyl-D-phenylalanyl-N-[(1S)-1-formyl-4-guanidinobutyl]-L-prolinamide,

((1-(2-((4-(aminoiminomethyl)benzoyl)amino)-3-(4-hydroxyphenyl)-1-oxopropyl)-4-piperidinyl)oxy)-(S)-acetic acid,

N-(2-(2-(((3-((aminoiminomethyl)amino)propyl)amino)carbonyl)-1-piperidnyl)-1-(cyclohexylmethyl)-2-oxoethyl)-(R,S)-glycine,

Ethyl 3-[[4-[[4-(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentynoate

(2-S-(n-Butylsulfonylamino)-3[4-(piperidin-4-yl)butyloxyphenyl]propionic acid hydrochloride, and

2(S)-[(p-Toluenesulfonyl)amino]-3-[[[5,6,7,8-tetrahydro-4-oxo-5-[2-(piperidin-4-yl)ethyl]-4H-pyrazolo-[1,5-a][1,4]diazepin-2-yl]carbonyl]-amino]propionic acid.

10. A method for reducing the risk of acute coronary ischemic syndrome in patients at risk to acute coronary ischemic syndrome comprising administering to the patient an effective amount of an antithrombotic agent and an effective amount of an angiogenesis promoter.

11. A method for reducing the risk of stroke in severe diffuse carotid vertebral disease or following carotid percutaneous transluminal coronary angioplasty which comprises administering to the patient an effective amount of an antithrombotic agent and an effective amount of an angiogenesis promoter.

12. A method for treating a patient having peripheral ischemic syndrome by administering to the patient an effective amount of an antithrombotic agent and an effective amount of an angiogenesis promoter.

13. A method for reducing the risk of acute peripheral ischemic syndrome in patients at risk to acute peripheral ischemic syndrome comprising administering to the patient an effective amount of an antithrombotic agent and an effective amount of an angiogenesis promoter.

14. The use of an antithrombotic agent, in combination with the use of an angiogenesis promoter, in the manufacture of a medicament effective for treating coronary ischemia.

The Patent Office

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Claims searched: 1-14

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Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.Q): A5B (BJA)

Int Cl (Ed.6): A61K 38/08, 38/18

Other: ONLINE: CAS-ONLINE, EPODOC, JAPIO, WPI

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
A	WO 97/35615A1 (MERCK & CO INC) See whole document, in particular claims 1 and 6-11	

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.